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Transfer Efficiency of Bacteria and Viruses from Porous and Nonporous Fomites to Fingers under Different Relative Humidity Conditions

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Fomites can serve as routes of transmission for both enteric and respiratory pathogens. The present study examined the effect of low and high relative humidity on fomite-to-finger transfer efficiency of five model organisms from several common inanimate surfaces (fomites). Nine fomites representing porous and nonporous surfaces of different compositions were studied. *Escherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis*, MS2 coliphage, and poliovirus 1 were placed on fomites in 10-μl drops and allowed to dry for 30 min under low (15% to 32%) or high (40% to 65%) relative humidity. Fomite-to-finger transfers were performed using 1.0 kg/cm^2 of pressure for 10 s. Transfer efficiencies were greater under high relative humidity for both porous and nonporous surfaces. Most organisms on average had greater transfer efficiencies under high relative humidity than under low relative humidity. Nonporous surfaces had a greater transfer efficiency (up to 57%) than porous surfaces (<6.8%) under low relative humidity, as well as under high relative humidity (nonporous, up to 79.5%; porous, <13.4%). Transfer efficiency also varied with fomite material and organism type. The data generated can be used in quantitative microbial risk assessment models to assess the risk of infection from fomite-transmitted human pathogens and the relative levels of exposure to different types of fomites and microorganisms.

Inanimate objects, or fomites, are a potential reservoir in the transmission of pathogens either directly, by surface-to-mouth contact, or indirectly, by contamination of fingers and subsequent hand-to-mouth, hand-to-eye, or hand-to-nose contact (1–5). Bodily fluids such as saliva, mucus, nasal secretions, blood, urine, and feces may all potentially contain pathogens that can be transmitted via fomites (6–9). A number of studies have shown that enteric and respiratory pathogens are capable of surviving from hours to months on fomites, depending on the numbers deposited, the type of microorganism, and the variable environmental conditions (10–12). Several studies have shown that inanimate surfaces found in day care centers (8, 13–17), schools (18), office buildings (19), homes (20–27), public areas (28), or hospitals (12, 29–33) can be reservoirs for secondary modes of transmission, with contaminated hands playing a critical role as a route of exposure.

The efficiency of transfer of a pathogen to the hand from the fomite is important in modeling the potential for its transmission (11, 34–36). This information can be used to understand the spread of disease in indoor environments and the potential for designing surfaces that reduce transfer efficiency and/or are antimicrobial (5). The purpose of this work was to better elucidate the transfer efficiencies of several different types of organisms under control conditions to provide data that may be used in quantitative microbial risk assessment (QMRA) models.

MATERIALS AND METHODS

Subjects. A single subject conducted the fomite-to-finger transfer experiments. Permission was obtained from the University of Arizona's Office for Human Subjects Research prior to the study.

Bacteria, virus, and preparation of inocula. (i) Study organisms. *Escherichia coli* (ATCC 15597), *Staphylococcus aureus* (ATCC 25923), *Bacillus thuringiensis* (ATCC 10792), and coliphage MS2 (ATCC 15597-B1) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Poliovirus 1 (PV-1; strain LSc-2ab) was obtained from the Department of Virology and Epidemiology at the Baylor College of Medicine (Houston, TX). These organisms were selected as model organisms for pathogenic Gram-negative and Gram-positive bacteria, spore-forming bacteria, and viruses.

(ii) Gram-negative and Gram-positive bacterial inoculum preparation. Frozen aliquots of *E. coli* and *S. aureus* were transferred into separate flasks containing 100 to 150 ml of tryptic soy broth (TSB; EMD, Gibbstown, NJ), incubated for 18 ± 2 h at 37°C on an orbital shaker (150 to 180 rpm), and streaked for isolation onto tryptic soy agar plates (TSA; EMD, Gibbstown, NJ). The bacteria were then subcultured in a flask of TSB and incubated for 18 ± 2 h at 37°C on an orbital shaker (150 to 180 rpm) (37).

(iii) Endospore-forming bacterial inoculum preparation. *B. thuringiensis* spores was prepared as previously described with minor modifications (38). Briefly, spores were suspended in Difco sporulation media with supplements (DSM + S; Becton, Dickinson and Company, Sparks, MD), cultivated for 24 h at 37°C on an orbital shaker (150 to 180 rpm), and resuspended in fresh DSM + S to obtain a final optical density at 600 nm (OD_{600}) of 0.1 (Spectronic Genesys 5; Milton Roy, Ontario, Canada).

(iv) Virus inoculum preparation. MS2 coliphage was prepared as previously described with minor modifications (37). Briefly, 0.1 ml of phage suspension and 0.5 ml of a log-phase *E. coli* 15597 (host bacterium) culture were added to top agar, and the agar was melted and maintained at 48°C. The inoculated top agar was mixed and poured over the TSA. The solidified agar overlay plates were then inverted and incubated at 37°C for 24 h. TSB was then added to each plate and maintained at room temper-

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ature for 2 h. The TSB eluent was aspirated and centrifuged (1,989 × *g* for 10 min), after which the supernatant was filtered through 0.22-μm-pore-size Acrodisc syringe filters (Pall Corporation, Ann Arbor, MI) premoistened with 3% beef extract (Bacto; Becton, Dickinson and Company, Sparks, MD). The coliphage stock was titrated prior to storage at 4°C.

PV-1 propagation and plaque-forming assays were conducted as described previously (39, 40). Briefly, PV-1 was propagated on buffalo green monkey kidney (BGM) (ATCC CCL-81; American Type Culture Collection, Manassas, VA) cell line monolayers with minimal essential media (MEM) containing 5% calf serum (HyClone Laboratories, Logan, UT) at an incubation temperature of 37°C with 5% CO_2. Plaque-forming assays were performed using six-well plates with confluent monolayers of the BGM cells.

Control wash and disinfection. Prior to all experiments, the subject's hands were washed with warm water and nonantibacterial liquid dish detergent (Liquid Joy; Procter and Gamble, Cincinnati, OH) for 45 s, rinsed with water, and dried with paper towels. Each hand was then sprayed twice with 70% ethanol, rubbing the alcohol thoroughly over the hands and wrists for 15 s, and subsequently air dried. After conducting fomite-to-finger transfer experiments with the prepared inocula, fingers were disinfected twice with 70% ethanol, wrapped with a 70% ethanol-saturated paper towel for 30 s, washed and rinsed using warm water and Softsoap antibacterial liquid hand soap (Colgate-Palmolive, Morristown, NJ) for 45 s, and then dried with paper towels. After sampling of fingers for *B. thuringiensis* spores and PV-1 was performed, fingers were placed in 10% bleach (The Clorox Company, Oakland, CA) for 15 s and then neutralized with 10% sodium thiosulfate (EMD, Gibbstown, NJ). The hands were then washed as described above to prepare for subsequent trials. Up to four trials were performed on the same day, and no visible change of the skin condition was observed throughout the day of the experiments.

Relative humidity conditions and temperature. The study consisted of two relative humidity conditions—high (40% to 65%) and low (15% to 32%) relative humidity. To achieve both humidity conditions, two separate incubators were turned off and used. Incubator temperatures thus reflected room temperature ranges of 19°C to 25°C. The temperature and relative humidity were monitored with a high-accuracy Thermo-Hygrometer (VWR, Mississauga, Ontario, Canada). During days with higher ambient relative humidity in the laboratory, t.h.e. desiccant (EMD, Gibbstown, NJ) and Drierite desiccant (Drierite, Xenia, OH) were utilized in the incubator to decrease the relative humidity to the low relative humidity range (15% to 32%). Under laboratory ambient lower relative humidity conditions, a Bionaire humidifier (Milford, MA) was used in the specific incubator to increase the relative humidity to the high relative humidity range (40% to 65%).

Fomites tested. Nine different types of fomite materials (six nonporous and three porous) were tested that ranged in surface area from 16 to 25 cm^2 (Table 1). All fomites were sterilized by autoclave, with the exception of the acrylic material, which was subjected to radiation under UV light (254 nm) for 30 min on each side. After fomite-to-finger transfers performed with *E. coli*, *S. aureus*, and MS2, nonporous fomites were sprayed three times with 70% ethanol and allowed to dry for 10 min. Nonporous fomites used in the finger transfers with *B. thuringiensis* spores and PV-1 were disinfected with 10% bleach (The Clorox Company, Oakland, CA), allowed to sit for 10 min, and subsequently neutralized in 10% sodium thiosulfate (EMD, Gibbstown, NJ). Fomites were then washed under warm running water with nonantibacterial soap (Liquid Joy; Procter and Gamble, Cincinnati, OH), rubbed with a wet paper towel on the surface area of the inoculation, rinsed thoroughly with reverse-osmosis (RO)-treated water, air-dried, and autoclaved. Cotton and polyester fomites were discarded after use. Paper currency was autoclaved and reused.

Inoculation of fomites. (i) Layout of fomites. For each of the six nonporous and three porous fomites, seven swatches were evenly spaced on the middle shelf of an incubator. Each trial consisted of three control swatches, three fomite-to-finger transfer swatches, and one negative-control swatch to ensure that the fomites had not been previously contaminated.

TABLE 1 Fomites tested

Fomite	Description	Manufacturer or source
Nonporous		
Acrylic	Poly-methyl methacrylate, matte nonglare finish	Home Depot, Atlanta, GA
Glass	Slides	VWR, Mississauga, Ontario, Canada
Ceramic tile	Porcelain	Home Depot, Atlanta, GA
Laminate	Various colors	Wilsonart International, Temple, TX
Stainless steel	Gauge 304	AK Steel Corporation, West Chester, OH
Granite	One from India and two from Brazil	Granite Kitchen & Bath Countertops, Tucson, AZ
Porous		
Cotton	100% of fabric content	Hometrends brand, Walmart, Bentonville, AR
Polyester	100% of fabric content	Hometrends brand, Walmart, Bentonville, AR
Paper currency	Cotton-based one-dollar bills	Bureau of Engraving and Printing, Fort Worth, TX, or Washington, DC

(ii) Organism concentration. The concentration of organisms added to the fomites was approximately 10^7 to 10^8 CFU/cm^2 of *E. coli* in TSB (EMD, Gibbstown, NJ), 10^8 to 10^9 CFU/cm^2 of *S. aureus* in TSB, 10^6 to 10^7 CFU/cm^2 of *B. thuringiensis* spores in DSM + S (Becton, Dickinson and Company, Sparks, MD), 10^9 to 10^{11} PFU/cm^2 of MS2 in TSB, and 10^8 PFU/cm^2 of PV-1 in phosphate-buffered saline (PBS) (Sigma-Aldrich, St. Louis, MO) in 10-μl droplets. Using a pipet tip, the 10-μl inoculum droplets were spread over an approximately 1-cm^2 area on the center of each fomite. The paper currency was divided into four 24-cm^2 sections; two one-dollar bills were used to make the seven swatches. With paper money for each set of transfer experiments, a new 1-cm^2 area on each of the 24-cm^2 sections was inoculated. Using an ink marker, an identifying spot was placed near the inoculated area on cotton, polyester, and paper money due to the absorbance. The fomites were allowed to dry for 30 min. Under high relative humidity conditions, nonporous fomites were visibly moist, while under low relative humidity conditions, they were visibly dry.

Fomite-to-finger transfer, sampling, and assays. (i) Fomite sampling. Fomites were sampled using a cotton-tipped swab applicator (Puritan Medical Products Company, Guilford, ME) after inoculation with *E. coli*, *S. aureus*, *B. thuringiensis*, and MS2. In the case of PV-1, a polyester fiber-tipped applicator swab (Falcon; Becton, Dickinson and Company, Cockeysville, MD) was used. Swabs were wetted in 1.0 ml of PBS (Sigma-Aldrich, St. Louis, MO), and then an area of approximately 6 cm^2 on the fomite was swabbed using a firm sweeping and rotating motion to ensure that the entire seeded surface area (approximately 1 cm^2) was swabbed. The swab was then placed back into the remaining PBS and vortexed for 5 s.

(ii) Transfer experiment. One transfer trial consisted of three separate fomite-to-finger transfer events using the index, middle, and ring fingers of the right hand for each surface type. Two transfer trials were conducted, resulting in six transfers in total for each fomite under both low and high relative humidity. A protocol adapted from Ansari et al. (41) and Mbithi et al. (42) was used to perform the fomite-to-finger transfer of test organisms from the previously mentioned nonporous and porous fomites to hands after a 30-min drying time. To assess transfer, the fomite was placed at the center of a scale with a digital readout, and a finger transfer was performed by placing the right hand finger on the center, covering the inoculated area of the fomite, for 10 s with 1.0 kg/cm^2 (98.0665 kPa) of average pressure (range, 700 g/cm^2 to 1,500 g/cm^2) (41, 42).

(iii) Finger sampling. Using a cotton-tipped swab applicator (Puritan Medical Products Company, Guilford, ME) moistened in 1.0 ml of PBS

TABLE 2 Fomite-to-finger transfer efficiency of microorganisms under low relative humidity of 15% to 32%

Surface type	Avg % transfer efficiency ± SD (range)[a]			
	E. coli	*S. aureus*	*B. thuringiensis*	MS-2
Nonporous				
Acrylic	40.7 ± 37.7 (6.4 to 93.5)	3.4 ± 2.5 (0.9 to 8.0)[c]	57.0 ± 12.0 (45.8 to 74.8)	21.7 ± 15.0 (3.0 to 40.6)[c]
Glass	5.1 ± 5.4 (0.7 to 15.1)[c]	20.3 ± 33.4 (0.6 to 85.4)	<0.5 ± 0.2 (<0.3 to 0.9)[b,c]	19.3 ± 13.2 (2.9 to 40.5)[c]
Ceramic tile	11.6 ± 11.8 (0.1 to 33.3)[c]	2.7 ± 2.3 (0.8 to 6.7)[c]	<0.2 ± 0.1 (<0.1 to 0.4)[b]	7.1 ± 4.0 (3.8 to 15.0)[c]
Laminate	21.7 ± 23.9 (5.2 to 66.5)	4.3 ± 2.4 (1.3 to 7.4)[c]	<0.2 ± 0.1 (<0.1 to 0.3)[b,c]	5.4 ± 3.6 (1.0 to 10.0)[c]
Stainless steel	3.8 ± 2.5 (1.5 to 7.1)[c]	4.0 ± 4.0 (1.1 to 11.9)[c]	<0.5 ± 0.2 (<0.4 to <1.0)[b,c]	6.9 ± 8.9 (1.4 to 24.2)[c]
Granite	<7.3 ± 10.6 (<0.1 to 28.0)[b]	3.9 ± 5.0 (0.7 to 13.9)	<0.04 ± 0.03 (<0.02 to 0.1)[b]	10.2 ± 5.0 (4.8 to 16.9)
Porous				
Cotton	<6.8 ± 7.0 (<0.3 to <15.4)[b]	<1.0 ± 0.6 (<0.4 to <1.9)[b]	<0.6 ± 0.1 (<0.5 to <0.8)[b]	0.03 ± 0.02 (0.01 to 0.1)
Polyester	<0.37 ± 0.28 (<0.08 to <0.9)[b]	<0.37 ± 0.48 (0.04 to 1.3)[b]	<0.6 ± 0.6 (<0.2 to <1.7)[b]	0.3 ± 0.2 (0.1 to 0.7)[c]
Paper currency	<0.05 ± 0.04 (<0.02 to 0.1)[b]	0.2 ± 0.1 (0.1 to 0.4)	<0.1 ± 0.1 (<0.02 to 0.2)[b]	0.4 ± 0.4 (0.1 to 0.9)

[a] % transfer efficiency = (CFU or PFU finger/CFU or PFU control fomite) × 100 ($n = 6$ for each fomite and microorganism).
[b] Transfer of organisms from fomite to fingers for one or more transfer events was below the detectable limit of 10 CFU/2 cm^2 (indicated by <).
[c] There was a statistically significant difference (Student's t test; $P \leq 0.05$) in the transfer efficiency results between low and high relative humidity conditions.

(Sigma, St. Louis, MO), the index, middle, and ring finger pads were sampled using a sweeping and rotating motion. Subsequently, the swab applicator was placed in the PBS vial and vortexed. A polyester-tipped swab (Puritan Medical Products Company, Guilford, ME) was used to sample PV-1.

(iv) Organism assays. *E. coli*, *S. aureus*, and *B. thuringiensis* spores were enumerated using the spread plate technique on MacConkey agar (EMD Chemicals Inc., Gibbstown, NJ), mannitol salt agar (MSA; EMD Chemicals Inc., Gibbstown, NJ), and TSA (EMD Chemicals Inc., Gibbstown, NJ) plates, respectively. The plates were incubated at 37°C for 18 ± 2 h. *B. thuringiensis* spore samples were heat shocked at 81 ± 2°C for 10 min prior to spread plating to stimulate germination. The MS2 plaque assay was conducted using the double-agar overlay method and TSA (EMD Chemicals Inc., Gibbstown, NJ) (39, 43). PV-1 titrations were performed using 10-fold-serial-dilution plaque-forming assays as described previously (39, 40). All dilutions were assayed in duplicate.

Transfer efficiency calculation and statistical analyses. (i) Calculation of transfer efficiency. Bacterial colonies and viral plaques were enumerated and the transfer efficiencies were calculated using equation 1 below (44, 45). The transfer efficiency (TE) is defined as the number of CFU or PFU recovered from finger relative to the CFU or PFU recovered from the control fomite. If bacteria or phage were not recovered from the finger pad, the lower detection limit of 10 CFU or PFU was used as an estimate for the amount of microorganism recovered as previously described (46, 47). A less-than sign (<) is used to indicate that the transfer efficiency was lower than the lower limit of detection.

$$\text{TE}\,(\%) = \left(\frac{\text{CFU or PFU finger}}{\text{CFU or PFU control fomite}}\right) \times 100$$

Finger transfer values greater than 100% were truncated to 100% because, based on this formula, the TE could be greater than 100% when the microbial recovery efficiency from the finger is greater than that from the control fomite.

(ii) Statistical analyses. Data were entered in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) and the software package StatPlus: mac, 2009 (AnalystSoft), to compute the descriptive statistic measures of mean percent transfer efficiency, the standard deviation, and statistical significance. Student's t test was performed to determine whether there was a statistically significant difference in percent transfer efficiency of a particular type of microorganism between low and high relative humidity conditions. Differences were considered statistically significant if the resultant P value was 0.05 or lower.

RESULTS

Influence of relative humidity on microbial transfer. The percent transfer efficiency was determined for 468 fomite-to-finger transfers; 234 transfer events were performed under both low and high relative humidity conditions. Tables 2, 3, and 4 summarize the fomite-to-finger percent transfer efficiency results. Relative humidity influenced the transfer rate of pathogens from fomites to fingers except for PV-1. Most organisms had greater transfer efficiencies under high relative humidity (<0.1% to 79.5%) than under low relative humidity (0.03% to 57%), with a few exceptions (Tables 2 and 3). *E. coli* and MS2 had greater transfer efficiencies under high relative humidity for all the fomites. *S. aureus* had greater transfer rates under high relative humidity except for cotton and paper currency. *B. thuringiensis* spores had greater transfer rates under high relative humidity except for paper currency. PV-1 seemed not to be influenced by relative humidity, with transfer efficiencies for ceramic tile, laminate, and granite under low relative humidity of 23.1%, 36.3%, and 33.8%, respectively, compared to 29.2%, 25.5%, and 25.9% under high relative humidity (Table 4).

Whether there was a statistically significant difference ($P < 0.05$) in transfer efficiencies between low and high relative humidity for each microorganism is shown in Tables 2 and 3. No statistically significant difference ($P > 0.05$) in transfer efficiencies between low and high relative humidity was observed for PV-1 (Table 4).

Influence of fomite type on microbial transfer. Fomite type did influence the transfer efficiency of all model organisms except PV-1. In general, the average transfer efficiencies were greater from nonporous surfaces (<0.04% to 57% under low relative humidity and 12.8% to 79.5% under high relative humidity) than from porous surfaces (<6.8% under low relative humidity and <13.4% under high relative humidity) (Tables 2 and 3). Under low relative humidity, acrylic provided the highest transfer rate for *E. coli*, *B. thuringiensis*, and MS2 coliphage (40.7%, 57%, and 21.7%, respectively), while *S. aureus* had the highest transfer efficiency from glass (20.3%) (Table 2). Under high relative humidity, glass provided the highest transfer rate for *E. coli* (78.6%), laminate for *S. aureus* (61.9%), and acrylic for *B. thuringiensis* and

TABLE 3 Fomite-to-finger transfer efficiency of organisms under high relative humidity of 40% to 65%

Surface type	Avg % transfer efficiency ± SD (range)[a]			
	E. coli	*S. aureus*	*B. thuringiensis*	MS-2
Nonporous				
Acrylic	53.3 ± 27.5 (30.4 to 98.0)	47.2 ± 17.9 (24.4 to 67.3)[d]	65.6 ± 15.9 (48.8 to 94.9)	79.5 ± 21.2 (54.1 to 100)[c,d]
Glass	78.6 ± 27.1 (38.0 to 100)[c,d]	45.5 ± 15.5 (25.7 to 65.5)	<33.8 ± 24.0 (<4.3 to 65.9)[b,d]	67.3 ± 25.0 (37.4 to 96.9)[d]
Ceramic tile	60.7 ± 45.4 (3.7 to 100)[c,d]	54.7 ± 18.8 (27.7 to 77.6)[d]	<21.2 ± 28.2 (<1.3 to 76.4)[b]	41.2 ± 18.8 (18.7 to 74.7)[d]
Laminate	27.4 ± 30.2 (1.9 to 77.0)	61.9 ± 24.7 (30.9 to 89.8)[d]	53.5 ± 19.6 (33.8 to 79.0)[d]	63.5 ± 24.0 (36.2 to 100)[c,d]
Stainless steel	54.1 ± 23.5 (29.4 to 99.0)[d]	48.3 ± 25.4 (16.6 to 85.5)[d]	57.0 ± 9.7 (47.5 to 71.4)[d]	37.4 ± 16.0 (19.5 to 62.4)[d]
Granite	36.5 ± 39.3 (0.3 to 100)[c]	39.6 ± 41.5 (1.3 to 100)[c]	12.8 ± 19.8 (0.1 to 42.7)	30.0 ± 24.3 (4.9 to 59.3)
Porous				
Cotton	<13.4 ± 11.7 (<2.6 to <33.3)[b]	<0.5 ± 0.5 (0.1 to 1.3)[b]	<3.5 ± 3.5 (<0.9 to <10.0)[b]	0.3 ± 0.3 (0.04 to 0.6)
Polyester	<0.7 ± 0.8 (<0.1 to <2.2)[b]	5.0 ± 6.9 (0.1 to 15.5)	<4.6 ± 6.1 (<1.1 to <16.3)[b]	2.3 ± 0.8 (1.2 to 3.2)[d]
Paper currency	<0.1 ± 0.3 (<0.01 to 0.7)[b]	0.2 ± 0.1 (0.1 to 0.3)	<0.1 ± 0.1 (<0.03 to <0.2)[b]	0.7 ± 0.5 (0.1 to 1.5)

[a] % transfer efficiency = (CFU or PFU finger/CFU or PFU control fomite) × 100 ($n = 6$ for each fomite and microorganism).
[b] The value for the transfer of organisms from fomite to fingers for one or more transfer events was below the detectable limit of 10 CFU/2 cm^2 (indicated by <).
[c] The value for the transfer of organisms from fomite to fingers for one or more transfer events was >100% and was truncated to 100%.
[d] There was a statistically significant difference (Student's t test; $P \leq 0.05$) in the transfer efficiency results between low and high relative humidity conditions.

MS2 coliphage (65.6% and 79.5%, respectively) (Table 3). PV-1 transfer did not seem to be influenced by the type of nonporous surfaces (Table 4). The transfer efficiency was least with paper currency under both low and high relative humidity conditions for *E. coli*, *S. aureus*, and *B. thuringiensis* (<0.05%, 0.2%, and <0.1% under low relative humidity and <0.1%, 0.2%, and <0.1% under high relative humidity, respectively). Cotton produced the lowest transfer efficiencies for MS2 under both low and high relative humidity conditions (0.03% and 0.3%, respectively).

DISCUSSION

The goal of this study was to obtain detailed quantitative information on fomite-to-finger transfer that could be used to model the probability of infection from exposure to various types of pathogens, a parameter needed for quantitative microbial risk assessments (1, 5, 48, 49). Unfortunately, there are no standard methods for quantifying transfer rates, making it difficult to compare the results from various studies. In addition, a number of factors affect microbial fomite-to-finger transfer efficiency: for example, washed versus unwashed hands (47), hands of different subjects, fomite type, relative humidity, and type of microorganism. In the present study, we focused on fomite type, relative humidity, and type of microorganism as parameters to evaluate and our results indicate that whereas transfer is influenced by the relative humidity and fomite type, different organisms differ greatly in the relative influences of these environmental factors. We conducted these experiments under clean finger conditions, but a previous study that conducted transfers under washed- and unwashed-hand conditions found that unwashed hands resulted in greater fomite-to-finger microbial transfer efficiencies. Therefore, under unwashed-hand conditions, which is more likely to be a real-world scenario, the transfer efficiency could be greater than that obtained in the present study. Incorporating more volunteer hands could also help to better characterize the effects of individual hand characteristics such as pH (50). However, the main focus of this study was not to determine the distribution of fomite-to-finger microbial transfer efficiencies that result from different subjects but rather to obtain a clearer picture of the transfer efficiencies for several nonporous and porous fomites under different relative humidity conditions using several types of microorganisms.

TABLE 4 Fomite-to-finger transfer efficiency of poliovirus 1

Surface type	Avg % transfer efficiency ± SD (range)[a]
Low (15% to 32%) RH[c]	
Ceramic tile	23.1 ± 24.0 (0.4 to 52.7)
Laminate	36.3 ± 8.7 (24.1 to 50.0)
Granite	33.8 ± 40.4 (0.4 to 100)[b]
High (40% to 65%) RH	
Ceramic tile	29.2 ± 6.4 (19.4 to 35.4)
Laminate	25.5 ± 15.5 (3.4 to 50.0)
Granite	25.9 ± 4.1 (19.7 to 32.1)

[a] % transfer efficiency = (PFU finger/PFU control fomite) × 100 ($n = 6$ for each fomite and microorganism).
[b] The value for the transfer event was >100% and was truncated to 100%.
[c] RH, relative humidity.

Five types of microorganisms, *E. coli*, *S. aureus*, *B. thuringiensis*, poliovirus, and MS2, were used in the present study as model microorganisms. These microorganisms have been widely used as models of Gram-positive and -negative bacteria, spore-forming bacteria, and enteric viruses. In particular, *E. coli*, *S. aureus*, and MS2 have been used in transfer studies (47, 51–54).

We used a cotton-polyester swab method that has been commonly used to recover microorganisms from fingers and fomites in transfer studies (37, 47), although other methods such as glove juice and rinsing in eluent (direct elution methods) have also been developed and utilized in some studies (41, 46, 52–54). We compared the recovery efficiencies of *E. coli* from finger using the swab method and a direct elution method described by Ansari et al. (41) and observed comparable efficiencies (data not shown). The swab method was selected in the present study because it is simpler and less labor-intensive than the direct elution method. Although a direct elution method may be more efficient in recovering microbes from porous fomites (44, 52, 53), we used the swab method for all nonporous and porous fomites in order to make the recovery method consistent across all types of surfaces (fomites and fingers).

The relative humidity greatly influenced the transfer for most of the microorganisms except for PV-1; however, on average, most had greater transfer rates at a high relative humidity. Under low relative humidity conditions, *E. coli*, MS2, and PV-1 had the greatest transfer efficiencies. No substantial difference of transfer efficiencies between different organisms was observed at a high relative humidity, although *E. coli* and MS2 exhibited slightly greater transfer rates than the other organisms. *B. thurigiensis* spores were poorly transferred under low relative humidity conditions from all surfaces except acrylic. Desiccation did not seem to account for these differences since *E. coli* is more influenced by desiccation than *S. aureus* and endospores (54). These differences may also reflect differences in the composition of the cell or endospore outer surface and in the hydrophobicity or other chemical/structural characteristics of the organisms. Generally, it would appear that the smoother surfaces provide greater transfer efficiencies.

As might be expected, porous surfaces had much lower transfer rates than the nonporous surfaces. Porous surfaces may entrap organisms within their matrix and provide a much greater surface area for attachment. Overall, acrylic tended to exhibit greater transfer, especially under low relative humidity; acrylic (polymethyl methacrylate) is a transparent thermoplastic, often used as a lightweight or shatter-resistant alternative to glass and paints.

PV-1 transfer rates were similar to the ranges reported by Mbithi et al. for hepatitis A virus (42), Ansari et al. for rotavirus (41), and Paulson for feline calicivirus (45) (16% to 32%). However, it should be noted that different experimental conditions (i.e., drying time, contact time and pressure, friction, etc.) were used to assess these transfer efficiencies. It is ideal to conduct an experiment under the same conditions as the previous study. Although we applied some of the conditions used in a previous study such as inoculum volume, contact time, and pressure, the other conditions were not necessarily the same as the previous studies since each study used different experimental conditions (for these conditions, we chose the parameters that are considered most reasonable for our particular study).

Rheinbaben et al. (55) found lower transfer of ΦX174 coliphage from door handles to hands of volunteers (0.001% to 0.4%) than that of MS2 from stainless steel (37.4%) under high relative humidity observed in the present study. Rusin et al. (37) found PRD 1 phage to have transfer efficiencies of 33.5% and 65.8% from faucet handle and phone receiver, respectively. Julian et al. (47) investigated the effects of washed and unwashed hands on the transfer rates of MS2, ΦX174, and coliphages from glass slides to fingers and fingers to glass slides and found similar transfer rates of 25%, 21%, and 37% for unwashed hands and 26%, 11%, and 39% for washed hands, respectively. The fomite-to-finger transfer efficiency of MS2 observed in our study agrees with the findings of these previous reports. Transfer rates for MS2 under high relative humidity for glass were greater (67.3%) than under low relative humidity (19.3%) (Tables 2 and 3). Transfer rates for porous fomites, cotton, polyester, and paper currency under low and high relative humidity were also comparable to the PRD 1 transfer rates found by Rusin et al. (37).

Previous studies have used the 1.0 kg/cm^2 pressure value which has been estimated to be equivalent to the pressure applied in a handshake without friction or to opening a door with a door handle with friction (6, 41, 42). Other studies have used lower levels of contact pressure to simulate children handling and grasping objects (47) or to represent ordinary touching of environmental surfaces (42). Mbithi et al. (42) found a significant difference in the amounts of pressure and friction applied in determining the amount of hepatitis A virus transferred from stainless steel disks to fingers. Different contact pressures and times could have been examined, which can provide a broader view of the transfer rates under various contact pressures and times.

Another factor that might influence the results is drying time. We selected a 30-min drying time based on our preliminary studies. The seeded suspension medium on various surfaces became visibly dry between 15 min to 32 min under a relative humidity range of 20% to 34%, which allowed us to compare the effects of low and high relative humidity on fomite-to-finger transfer efficiencies. Other investigators have used drying times ranging from 5 min to 48 h and contact times ranging from 5 s to 30 s (6, 37, 41, 42, 45–47, 52, 54–58). Based on these previous studies, we could expect the transfer rates to be lower over longer drying periods, allowing the seeded suspension medium to become drier, which resulted in lower transfer rates.

The transfer efficiencies observed in our study for *S. aureus* from nonporous surfaces under high relative humidity (39.6% to 61.9%) were greater than what was reported by Scott and Bloomfield (9% to 43%) (54). The difference in contact time and drying time could have influenced the different transfer rates. Our low relative humidity transfer efficiencies are similar to what Scott and Bloomfield (54) reported at the 24-h drying time of 9.05%. Transfer efficiencies for porous fomites under low and high relative humidity were lower than those reported by Scott and Bloomfield (54) and Sattar et al. (53), with the exception of polyester at a high relative humidity (5% transfer). Sattar et al. (53) observed the same greater transfer efficiency of *S. aureus* from poly-cotton fabric than from 100% cotton and explained that this might have been due to the hydrophobic nature of polyester material being higher than that of cotton. The hydrophobic nature reduced the ability of the bacterial cells to penetrate deeper into individual fibers. Data reported by Hubner et al. (51) from a study on the hand-to-paper-to-hand transfer cycle of *E. coli* (0.009%) agree with our fomite-to-finger observations of *E. coli* with paper currency under low relative humidity.

A possible reason for the higher transfer efficiency under high relative humidity observed in our study was that the high humidity prevented the inoculum from drying, resulting in greater transfer efficiencies. Previous studies have shown that virus transfer efficiencies between contaminated surfaces were greater when the seeded suspension medium was not completely dry (4, 7, 41, 42, 58, 59). This was seen with coliphage MS2, which had the most significant difference between low and high relative humidity with the nonporous inanimate surfaces and to a lesser degree with the porous surfaces. It was also seen with Gram-negative *E. coli*, Gram-positive *S. aureus*, and spores of *B. thuringiensis*. In most cases, there was a significant ($P \leq 0.05$) difference between nonporous and porous surfaces with respect to organism transfer efficiencies.

The transfer efficiency for each organism on a specific surface can be influenced by the seeded suspension medium along with the physicochemical properties of the species and how it interacts with the physical properties of the environmental surfaces (60–63). Both the isolectric point and the hydrophobicity of the surface can influence the interactions between the fomite and the organism. MS2 has a relatively low isoelectric point of pH 3.9

compared to many nonenveloped enteric viruses and is often less attracted to common fomites (62, 64). In the case of the low transfer efficiencies seen with *B. thuringiensis* spores and *S. aureus* from nonporous surfaces under low relative humidity, a possible explanation could be that the spores and the Gram-positive bacteria had stronger electrostatic interactions through van der Waals forces, resulting in a stronger attachment to the fomites (61).

In the present study, a number of different fomites were tested, providing a broad data set to estimate the distributions in the transfer efficiency that depend on the type of fomite and the microorganism. Our results highlight the importance of relative humidity in the organism fomite-to-finger transfer efficiency rates. Most species had greater transfer efficiencies under high relative humidity than under low relative humidity. The fomite type was found to influence the transfer efficiency, with nonporous surfaces having greater transfer efficiency than porous surfaces. The outcomes of our study are beneficial to industries focusing on infection control and prevention as well as the academic field of public health by providing fomite-to-finger transfer efficiencies as input values for exposure assessment in QMRA models.

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